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A prolonged dry season and nitrogen deposition interactively affect CO_2 fluxes in an annual Mediterranean grassland

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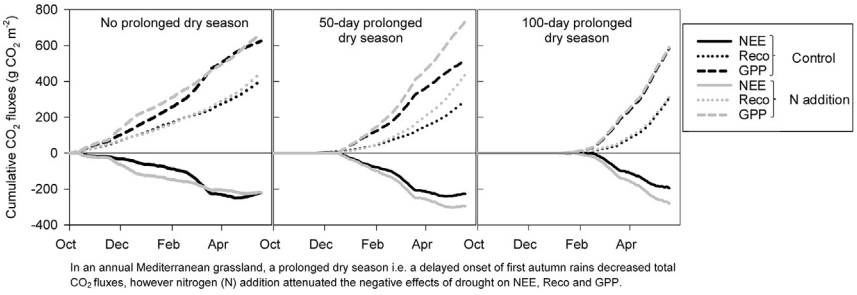
HIGHLIGHTS

- Annual Mediterranean grassland communities are sensitive to a prolonged dry season.
- A prolonged dry season decreased ecosystem CO_2 fluxes.
- Nitrogen attenuated the effects of a prolonged dry season on total CO_2 fluxes.
- A prolonged dry season affected productivity and function group proportion.

GRAPHICAL ABSTRACT

In an annual Mediterranean grassland, a prolonged dry season i.e. a delayed onset of first autumn rains decreased total CO_2 fluxes, however nitrogen (N) addition attenuated the negative effects of drought on NEE, Reco and GPP.



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ABSTRACT

Mediterranean annual grasslands are species-diverse ecosystems of high economic and ecological value. CO_2 and water fluxes in these grasslands are triggered by the first rains in autumn, after a long hot and dry summer. Climate change scenarios project altered rainfall patterns, such as prolonged dry season into the autumn, while simultaneously nitrogen (N) deposition is increasing globally. However, how these global change drivers will interact to affect Mediterranean grassland CO_2, water fluxes and productivity is still unclear.

In a greenhouse experiment, we subjected the seedbank of an annual Mediterranean grassland to a factorial treatment, by prolonging the dry season by 0 days (i.e. no autumn drought), 50 days and 100 days and crossing these drought treatments with two levels of N deposition: no N and N addition.

A delayed onset of the rain season, i.e., a prolonged dry season, induced lower CO_2 and water fluxes throughout the growing season and a lower aboveground biomass by the end of the study period. However, N addition attenuated the effects on NEE, Reco and GPP, but did not affect aboveground biomass or functional group composition. A prolonged dry season also lowered the productivity of forbs, the dominant functional group in our grassland. Our results anticipate important effects of interacting global change drivers on Mediterranean grassland functioning.

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1. Introduction

Grasslands occupy approximately 40% of the earth surface, thereby playing an important role in the CO_2 and water exchange with the atmosphere (Aires et al., 2008a, 2008b; Suyker and Verma, 2001). In the Mediterranean Basin, open grasslands or understory grass communities

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in oak woodlands are biodiverse rich systems and important providers of ecosystem services, such as fodder production or CO_2 sequestration (Bugalho et al., 2011). However, droughts and increasing nitrogen (N) deposition, two important drivers of global change, are altering the structure and function of these grasslands (DeMalach et al., 2017; Harpole et al., 2007; Pereira et al., 2007; Zavaleta et al., 2003). Nonetheless, the magnitude of their effects, and in particular, their interactions remain largely unknown (Lei et al., 2016; Smith et al., 2016).

Mediterranean grasslands are dominated by annual C3 species, which typically germinate in autumn with the first major rain events (Espigares and Peco, 1995; Peco et al., 1998), reaching maximum growths in spring and senesce with the onset of the dry season (e.g. Xu and Baldocchi, 2004). These grasslands have a high inter-annual variability in productivity, CO_2 and water fluxes (Aires et al., 2008a, 2008b; Huxman et al., 2004a, 2004b; Jongen et al., 2011; Ma et al., 2007; Miranda, de Dios, et al., 2009; Pereira et al., 2007; Piayda et al., 2014) due mainly to the amount and seasonality of precipitation and occurrence of droughts, that also determine the length of the growing season (Huxman et al., 2004a; Jongen et al., 2011; Ma et al., 2007; Piayda et al., 2014). For instance, in a Mediterranean grassland in southern Portugal, during a dry year, total annual GPP (Gross Primary Productivity), ecosystem respiration and evapotranspiration were reduced by 58%, 55% and 34% respectively in comparison to a normal precipitation year (Aires et al., 2008a, 2008b).

Adding to climatic changes, N inputs from fossil fuel combustion and fertilization have also globally increased (Peñuelas et al., 2013). N is one of the major limiting nutrients affecting primary productivity (e.g. Cleland and Harpole, 2010; Vitousek and Howarth, 1991), which addition stimulates photosynthetic capacity and total leaf area and, thus positively may affect GPP (Niu et al., 2010; Pérez-Priego et al., 2015a; Guo et al., 2016). Simultaneously, N addition may also have a positive effect on ecosystem respiration (Luo and Zhou, 2006) which can counter balance effects on net CO_2 ecosystem exchange (NEE) (Niu et al., 2010). Nonetheless, N effects on primary productivity depend strongly on water availability (Xia and Wan, 2008). Mediterranean grasslands are frequently co-limited by water and N (Harpole et al., 2007; Hooper and Johnson, 1999), due to low precipitation and soil N content. However, studies addressing interactions between drought and N addition and its effects on grassland CO_2 and water exchange are scarce (Lei et al., 2016, but see; Guo et al., 2016; Harpole et al., 2007; Niu et al., 2009). Particularly, there are few studies addressing the importance of the delay in autumn precipitation (prolonged dry season), that determines the start of the grassland growing season, and N availability on Mediterranean grassland productivity and CO_2 and water fluxes.

Climate change models forecast, for the Mediterranean region, more frequent and prolonged droughts (e.g. Kovats et al., 2014). In effect, the Mediterranean Basin is already undergoing more frequent extreme drought events (Hoerling et al., 2012; Paredes et al., 2006; Trigo et al., 2013). Also, N inputs, for the Mediterranean Basin, are forecasted to increase from 7 kg N ha^{-1} yr^{-1} in the mid-1990s to 12 kg N ha^{-1} yr^{-1} by 2050 (Phoenix et al., 2006). However, the potential ecological effects of N deposition on Mediterranean ecosystems have received little attention (Bobbink et al., 2010, but see Bonanomi et al., 2006; Dias et al., 2011 and Ochoa-Hueso and Stevens, 2015), particularly using realistic forecasted N loads (Cleland and Harpole, 2010, but see Ochoa-Hueso and Manrique, 2010). Most studies addressing the effect of N deposition on ecosystems have used high N addition (e.g. 50–100 kg N ha^{-1} yr^{-1}) and few determined the interaction of N addition with drought (Smith et al., 2016).

The main goal of this study was to assess the consequences of a longer dry season and N deposition on CO_2 and water fluxes of a Mediterranean grassland. To achieve this goal, we conducted a greenhouse experiment with potted grassland communities, where the duration of the dry season and N addition (at the forecasted rate of 12 kg ha^{-1} yr^{-1}) were manipulated and chamber-based diurnal measurements of CO_2 and water fluxes performed.

We hypothesized that: (i) extending the dry season would lead to a reduction in total GPP and Reco, thereby decreasing the productivity of the grassland community; (ii) N addition would increase both GPP and Reco. As throughout the growing season, GPP should increase at a higher rate than Reco, N addition would thus attenuate negative effects of a prolonged dry season on NEE; (iii) a prolonged dry season and N addition would affect functional group composition in the grassland community; for instance, graminoid species may be favored to the detriment of slow growing forb species, thereby affecting ecosystem CO_2 fluxes.

2. Materials and methods

2.1. Study site and experimental setup

We conducted the experiment in a greenhouse situated at the School of Agriculture, University of Lisbon, Portugal, from 14th of October 2014 to 15th of May 2015. We used the top soil seed bank from a homogeneous semi-natural Mediterranean grassland, located in the *Companhia das Lezírias* (CL) estate, north-east from Lisbon (38°49′45.13″N, 8°47′28.61″W).

This grassland community was dominated by C3 annual species that germinate after first autumn rains (late September–October) and senesce with increasing temperatures at the onset of summer drought (mid-May–June) (e.g. Cerasoli et al., 2018). Dominant forb species were *Tolpis barbata* L., *Plantago bellardii* All. and *Tuberaria guttata* (L.) Fourr., dominant graminoids were *Agrostis pourretii* Willd., *Avena barbata* Link and *Gaudinia fragilis* (L.) P. Beauv., and dominant N-fixing forbs (i.e. legumes) were *Ornithopus compressus* L., *Trifolium arvense* L. and *Trifolium campestre* Schreb.

The climate is Mediterranean with hot and dry summers and mild winters. Mean annual temperature is 14.9 °C. Mean annual precipitation is 537 mm, approximately 84% of the precipitation falls between October and April. The soil is a well-drained deep Haplic Arenosol (IUSS Working Groups WRB, 2006) with a low water retention capacity and low total N content (0.18%) as also observed by Spehn et al. (2002), in a grassland site also at CL.

We collected thirty-five quadrats of 20 cm × 50 cm containing the first 3 cm of soil surface of the grassland community, in mid-September, avoiding tree canopies (Luzuriaga et al., 2005). Samples were brought to the laboratory, thoroughly homogenized, sieved through a 3 mm mesh, and air-dried at room temperature for 48 h; soil water content was approximately zero.

In the greenhouse, 48 PVC pots (29.5 cm inner diameter and 24 cm height) were filled up with homogenized and roughly sieved dry soil from the grassland site. A thin layer containing 1000 cm^3 of the well mixed surface soil containing the seedbank of our grassland community was spread on each pot. These pots had a perforated base for efficient water drainage, lined with a gardening screen cloth to avoid soil loss and wrapped with a white plastic film to avoid excessive heating.

Each of the 48 pots was assigned randomly to one of six treatments, with eight replicates. Considering a factorial design we prolonged the dry season by 0 days (i.e. no autumn drought), 50 days and 100 days and crossed these drought treatments with two levels of N deposition: (C-control or N-added). Specifically, treatments were, C0, C50, C100, N0, N50 and N100. For no drought (i.e. 0 days, the dry season was not prolonged) watering started on the 14th of October, for 50 days of prolonged dry season watering started on the 3rd of December and for 100 days of prolonged dry season watering started on the 20th of January.

N was added in the form of ammonium nitrate (NH_4NO_3) at the rate of 12 kg N ha^{-1} y^{-1} which is the averaged projected rate of atmospheric N deposition in the Mediterranean Basin for 2050 (Phoenix et al., 2006). Irrigation with distilled water (no N addition) or with N fertilizer solution (N addition treatment) was performed twice a week. From October to February watering regime followed the last ten years average amount and distribution of the study site (CL) (e.g. ranging from 99.7 mm in October to 54.6 mm in January), corresponding to no water limitation. However, as our objective was solely to determine the effect of a

prolonged dry season and not of a confounding drought effect along the growing season, soil was kept moist until the end of the experiment.

All pots were randomly distributed in the greenhouse and positions changed frequently in order to avoid potential differential temperature and light distribution in the greenhouse. In addition, eight smaller control pots were filled with sterilized soil to test for background seed contamination. But no contamination was observed during the period of the experiment. The greenhouse had no supplemental light (attenuation was ca. 16%) or temperature control, following the natural weather variation.

2.2. Microclimate

Microclimatic conditions inside and outside the greenhouse were continuously recorded (Fig. S1). Relative humidity and air temperature were recorded every 30 min (VP-3 humidity temperature and vapour pressure sensor by an EM50 data logger, Decagon Devices, Pullman, USA). Daily integrated photosynthetic photon flux density (PPFD) was obtained from a meteorological station at the School of Agriculture. Volumetric soil water content (SWC) was continuously measured every 30 min using EC-5 soil moisture sensors (Decagon Devices, Pullman, USA) installed in three pots per treatment.

2.3. Ecosystem CO_2 and water fluxes

Net grassland community CO_2 exchange (NEE), ecosystem respiration (Reco) and evapotranspiration (ET) were measured using portable gas exchange chambers (39.5 cm × 39.5 cm × 54 cm) linked in closed dynamic circuit to an infrared gas analyzer (IRGA LI-840, Lincoln, NE, USA), measuring CO_2 and water vapour. Gas exchange chambers were made of 3 mm thick transparent plexiglass (>95% light transmittance) to measure NEE and ET, and of opaque PVC, covered with an insulation layer of reflective aluminum foil to measure Reco. The chambers were tightly fitted to the pots and two small fans were fixed inside the chambers to continuously mix the air. A small pump circulated an air flow of 1 L min^{-1} through the sampling circuit and into an infrared gas analyzer (IRGA LI-840, Lincoln, NE, USA), which measures CO_2 and water vapour. Flux calculations and corrections for CO_2, water vapour dilution followed recommendations by Pérez-Priego et al. (2015b). Thus, for instance, as a procedure in all our measurements, the first 20 s after chamber closure were discarded; and we only considered values which reflected a steady increase/decrease in CO_2. Each measurement took no longer than 3 min.

As Reco measurements were done approximately within 1 min after NEE measurements, estimations of gross photosynthesis (GPP) were obtained by subtracting Reco from NEE. Diurnal time-courses (08 h30, 12 h00, and 16 h00 hours) were conducted monthly (except in April, due to bad weather conditions), on clear sky days, over the course of the growing season on the 18th of December, 9th of January, 9th of February, 10th of March and 11th of May on three pots per treatment. On the 12th of November and 21st of January measurements were only done at noon. Air temperatures inside and outside the chambers were also registered. At each flux measurement date, leaf area index (LAI) was recorded as described below.

Cumulative NEE, Reco and GPP, from 14th of October 2014 to 15th of May 2015, were calculated based on daily-integrated modeled CO_2 fluxes. We modeled NEE using a Michaelis–Menten rectangular hyperbola (e.g. Falge et al., 2001; Li et al., 2005):

$$NEE = \frac{\alpha \text{ x } NEEsat \text{ x } PPFD}{\alpha \text{ x } PPFD + NEEsat} + Reco \quad (1)$$

where α is the apparent quantum yield or the initial slope of the light response curve. NEEsat is the maxium NEE at light saturation (2000 μmol photons m^{-2} s^{-1} was used in this study) and Reco is ecosystem respiration.

An estimate of Reco was calculated by the Lloyd and Taylor (1994) equation (e.g. Falge et al., 2001; Reichstein et al., 2002):

$$Reco = Reco(ref) \text{ x } e^{E0\left(\frac{1}{Tref-T0}-\frac{1}{T-T0}\right)} \quad (2)$$

$Reco_{(ref)}$ is the ecosystem respiration a at reference temperature (Tref). Tref was set to 15 °C (288.15 K), the average temperature in our data set, as suggested by Reichstein et al. (2002). E0 and T0 were fixed at −46.02 °C (227.13 K) and 308.56 K as in the original Lloyd and Taylor (1994) model. T is air temperature (in K).

The parameter values, for both NEE and Reco, obtained for each measurement date (i.e. per month) were considered as the average value for that month. Measurements on the 9th and 21st of January were pooled together as an average value for the month of January. As we did not perform measurements during April, due to weather conditions, we used average model parameters of March and May. Hourly air temperatures and PPFD for the period from, 14th of October 2014 to 15th of May 2015, were then used in Eqs. (1) and (2) to estimate hourly Reco and NEE. Hourly GPP was subsequently calculated as:

$$GPP = Reco - (NEE)$$

Modeled and measured NEE and Reco values were significantly correlated ($P < 0.001$; Table S1).

2.4. LAI

LAI in each pot was estimated with five intercalar PAR readings above and below vegetation, using the external PAR sensor of the ceptometer (AccuPAR PAR/LAI Ceptometer Model LP-80; METER Group, Inc. USA). PAR readings were taken close to solar noon, approximately every fortnight. LAI was determined from PAR readings (Anderson, 1981) using a free software from the Meter Group (https://www.metergroup.com/environment/products/accupar-lp-80-leaf-area-index/). LAI estimates were only possible when plant height exceeded PAR sensor height (≈7 cm). LAI in May was corrected for senescence based on aboveground biomass.

2.5. Aboveground biomass

Aboveground biomass was determined on four replicates per treatment on two sampling dates (15th of April and 15th of May) ($n = 4$, total of 24 pots on each sampling date). All plants in the pots were clipped at ground level, separated into functional groups (forbs, graminoids and legumes) (see Nogueira et al., 2017 for more details). Biomass was oven dried at 60 °C for 48 h prior to weighing.

2.6. Statistical analysis

Statistical analysis was performed using Sigmaplot 13.0 (Systac Software, Inc., SanJose, USA) and IBM SPSS Statistics 23.0 (IBM Corp. Armonk, NY, USA). Dependent variables were tested for homogeneity of variances, normality and sphericity (for repeated measure analysis) and transformed whenever necessary. Significance level was set to $P = 0.05$. A three-way ANOVA, with number of days of prolonged dry season, N addition and month as factors, was used to analyze data on aboveground biomass and functional group proportion. A two-way ANOVA, with number of days of prolonged dry season and N addition as factors was used to analyze differences in LAI between treatments on each measurement date. Maximum NEE, Reco, GPP and ET flux rate values of the diurnal course (hereafter: NEE_{max}, $Reco_{max}$, GPP_{max} and ET_{max}) were analyzed using a repeated measure ANOVA, with date of measurement, number of days of prolonged dry season and N addition as factors. Tukey pairwise multiple comparison procedures were applied to determine individual differences between means. Linear

regressions were used to relate NEE_{max}, $Reco_{max}$, GPP_{max} and ET_{max} of each prolonged dry season treatment to LAI.

3. Results

3.1. Microclimate conditions

Average air temperature throughout the study was 15.4 °C, with October and May, being the hottest months with average temperature of 22.9 °C and 19.3 °C, respectively (Fig. 1). Maximum values in vapour pressure deficit (VPD) were also registered during those months and in March and April (Fig. 1). January and February were the coldest months with daily average temperatures of 11.9 °C and 12.0 °C, respectively (Fig. 1). After day 10 in February average air temperature begun to increase steadily, followed by daily integrated photosynthetic photon flux density (PPFD) that reached maximum values during April and May (Fig. 1). Microclimate conditions inside and outside the greenhouse followed the same pattern from 14th of October 2014 to 15th of May 2015. Average air temperature inside the greenhouse was 0.88 °C higher and relative humidity 3.15% lower compared to outside the greenhouse (Fig. S1).

Soils were dry (SWC of 1.05% ± 0.37, mean ± SE) for the 50 and 100 days watering delay treatments, before first watering events. Thereafter, SWC averaged 22.37% ± 0.92, which corresponded to 75% of the field capacity (≈30%), for all six treatments throughout the remaining growing season, with no substantial differences between all treatments (Fig. S2).

3.2. Seasonal variations in measured CO_2 and water flux rates

Maximum CO_2 flux rates (NEE_{max}, $Reco_{max}$ and GPP_{max}) were significantly ($P < 0.001$) affected by the prolonged dry season treatments (Table 1). However, direction and magnitude of response depended on date of measurement (Table 1; Fig. 2a, b and c). N addition did not have a significant effect on maximum CO_2 flux rates (Table 1).

A prolonged dry season significantly induced, until late January, lower flux rates of NEE_{max} (i.e. more positive values) and GPP_{max} compared to treatments with no prolonged dry season (C0 and N0) (Fig. 2b, c). C0 and N0 exhibited relatively constant fluxes of NEE_{max} and GPP_{max} throughout most of the growing season, with high NEE_{max} and GPP_{max} values in December and March (Fig. 2b, c). By February, NEE_{max} and GPP_{max} fluxes of communities that had been subjected to a 50-day prolonged dry season (C50 and N50) increased, becoming similar to C0 and N0 and significantly higher than C100 and N100 (NEE_{max} and GPP_{max}: $P < 0.001$) (Fig. 2b, c). In March, NEE_{max} and GPP_{max} flux rates of all six treatments were high with no significant differences between treatments (Fig. 2b, c). Interestingly, however, from March to May, the 100-day prolonged dry season had significantly higher NEE_{max}, and GPP_{max} flux rates compared to the other treatments that decreased during that time period (NEE_{max} and GPP_{max}, 0 days: $P < 0.001$; NEE_{max} and GPP_{max}, 50: $P = 0.001$ and $P = 0.009$, respectively) (Fig. 2b, c).

Until February, fluxes of $Reco_{max}$ of C0 and N0 were relatively stable and in general significantly higher compared to prolonged dry season treatments (Fig. 2a). At the beginning of January, $Reco_{max}$ of N50 peaked reaching similar values to C0 and N0 and was significantly higher than C50 on this measurement date ($P = 0.041$) (Fig. 2a). In May, $Reco_{max}$ flux rates of C100, N100 and N50 treatments were significantly higher than those of C0 and N0 (Fig. 2a).

Extending the dry season also significantly affected the seasonal patterns of ET_{max} fluxes ($F_{2,17} = 31.06$, $P < 0.001$), with the effect of prolonged dry season treatments depending on date of measurement ($F_{12,17} = 11.72$, $P < 0.001$) (Fig. 2d). However, N addition did not have a significant effect on ET_{max} fluxes. ET_{max} of C50 and N50 treatments, 8 days after germination in December, were already similar to those of C0 and N0. By February, all six treatments had similar ET_{max} and increased, reaching a maximum in May. Indeed, in May, the 100-day prolonged dry season reached higher ET_{max} rates, that were almost significantly higher than no prolonged dry season and 50-day prolonged dry season ($t = 2.55$, $P = 0.065$ and $t = 1.78$, $P = 0.095$, respectively).

Table 1
Statistical results of a three-way repeated measures ANOVA of the effects measurement date, level of prolonged dry season and N addition on maximum CO_2 flux rates (NEE_{max}, $Reco_{max}$, and GPP_{max}). *DF* (degrees of freedom); *F* values from the statistic ANOVA; *P* values < 0.05 were considered significant.

	DF	NEE_{max}		$Reco_{max}$		GPP_{max}	
		F	*P*	*F*	*P*	*F*	*P*
Date of measurement (T)	6	107.47	<0.001	101.01	<0.001	171.10	<0.001
Prolonged dry season (D)	2	96.01	<0.001	57.10	<0.001	109.75	<0.001
N addition (N)	1	0.06	0.805	0.04	0.855	0.002	0.961
T * D	12	43.71	<0.001	12.57	<0.001	49.74	<0.001
T * N	6	2.09	0.109	0.81	0.483	1.04	0.410
D * N	2	1.64	0.236	3.94	0.048	2.37	0.136

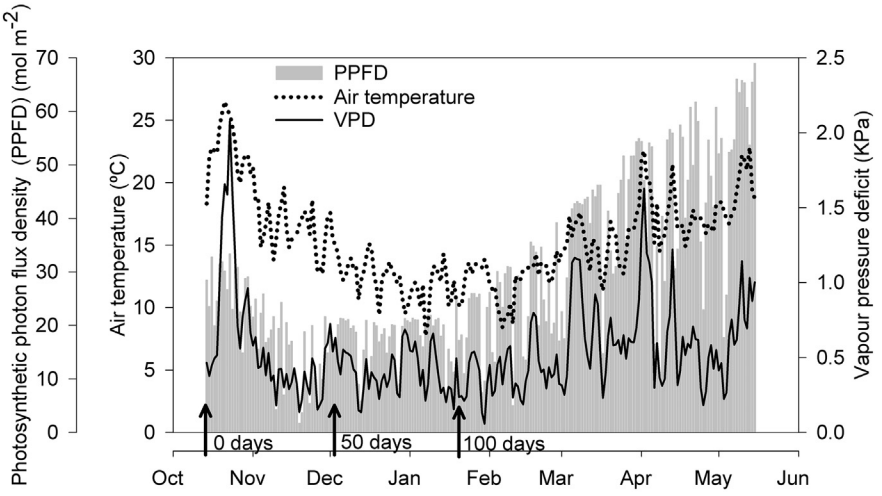

Fig. 1. Daily integrated photosynthetic photon flux density (PPFD) (mol m^{-2} d^{-1}), daily average air temperature (°C) and vapour pressure deficit (VPD) (kPa) over the course of the study. Arrows indicate date of first watering event for prolonged dry season treatments with (N0, N50, N100) and without N addition (C0, C50, C100), with watering starting on the 14th of October (0-day prolonged dry season); on the 3rd of December (50-day prolonged dry season); and 20th of January (100-day prolonged dry season).

3.3. Cumulative season CO_2 fluxes

Cumulative NEE, Reco and GPP, based on daily integrated modeled CO_2 fluxes (Fig. S3), over the course of the study period, are presented in Fig. 3a–c and Table 2. Extending the dry season reduced Reco by 35% and 42% and GPP by 21% and 23%, for 50 and 100- day prolonged dry season respectively (Fig. 3a–c; Table 2). NEE was reduced, particularly in the 100-day extended drought season (25% reduction). However, in both extended dry season treatments N addition attenuated the negative effects on NEE (Fig. 3c; Table 2). In communities that had been subjected to a 50-day prolonged dry season, N addition increased daily Reco, from March onwards, which translated into a 108.1 g CO_2 m^{-2} increment in cumulative Reco at the end of the study compared to C50, thus N50 treatments reached similar values to C0 at the end of the study (Fig. 3b; Table 2). Also, from February onwards, N addition had a positive effect on GPP resulting into a 172.6 g CO_2 m^{-2} increase in cumulative GPP compared to C50, and interestingly N50 reached higher values than the no prolonged dry season treatment (Fig. 3b; Table 2). As GPP increased at a higher rate than Reco (Fig. 3b) and earlier in the growing season (Fig. 3b), the grassland community subjected to this treatment (N50) was a stronger CO_2 sink compared to C50 and the other treatments (Table 2).

3.4. Development of LAI in relation to gas exchanges

LAI of C0 and N0 increased during winter reaching a LAI of 0.93 ± 0.06 (43% of its maximum LAI) in December. By March, C50 and N50 reached a similar LAI to that of the no drought treatment (Fig. 2e) and by April, all treatments had a similar LAI (Fig. 2e). Observed LAI values were well within the range of values for similar grassland communities (e.g. Aires et al., 2008a; Jongen et al., 2011). For all LAI measurement dates there was no effect of N addition.

For each prolonged dry season treatment changes in NEE_{max}, $Reco_{max}$, GPP_{max} and ET_{max} flux rates over the course of the growing

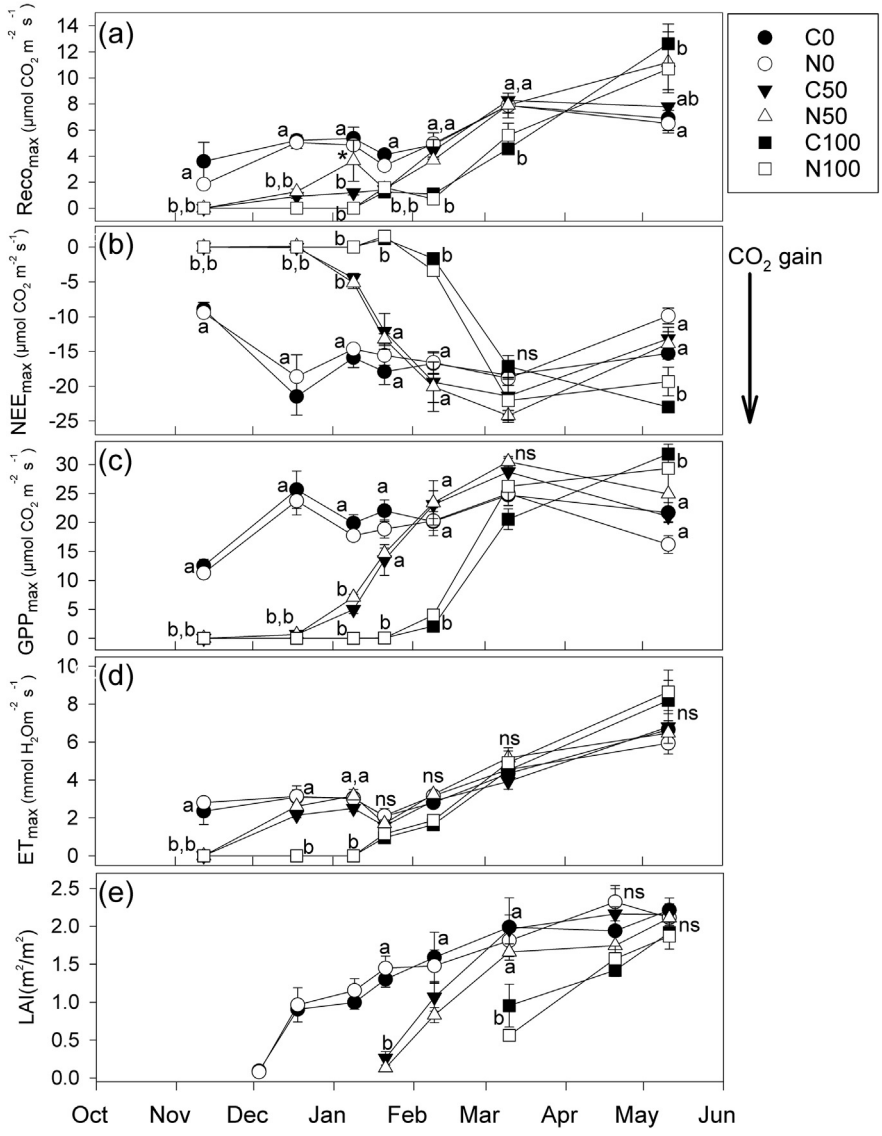

Fig. 2. Maximum measured CO_2 and water flux rates and LAI throughout the growing season determined for all treatments: (a) maximum ecosystem respiration flux rate ($Reco_{max}$); (b) maximum net ecosystem exchange flux rate (NEE_{max}); (c) maximum gross primary production flux rate (GPP_{max}), (d) maximum ecosystem evapotranspiration flux rate (ET_{max}) and (e) Leaf area index (LAI). C - represents no added nitrogen (control), N - represents added nitrogen, the number next to the letters represents number of days of extended dry season. Arrows at the top indicate date of first watering event, 14th of October (no prolonged dry season); 3rd of December (50-day prolonged dry season) and 20th of January (100-day prolonged dry season). Symbols show mean ± SE, $n = 3$. For each measurement date, different letters indicate significant differences between prolonged dry season treatments (Tukey's post hoc test, $P < 0.05$); ns refers to no significant difference between prolonged dry season treatments; asterisk (*) in $Reco_{max}$ flux rate in early January indicates a significant difference between C50 and N50.

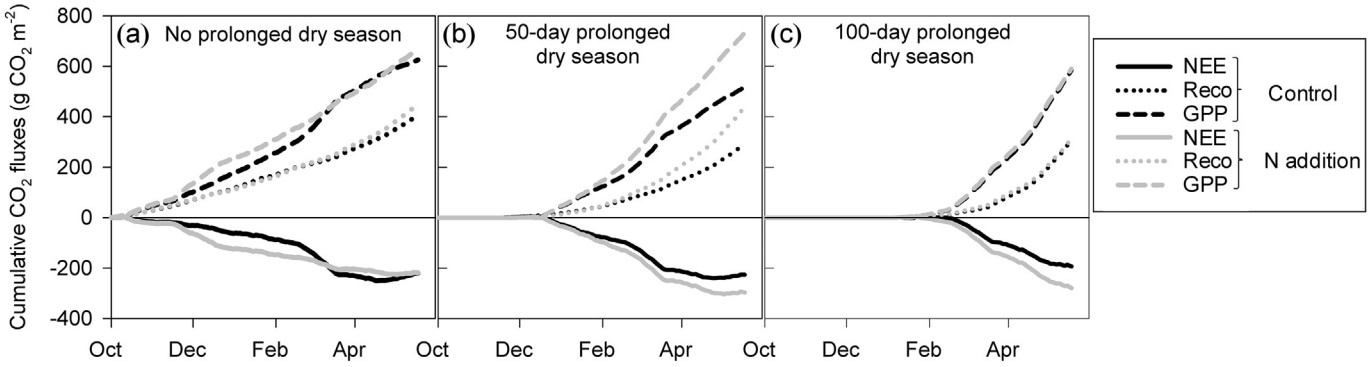

Fig. 3. Cumulative net ecosystem exchange (NEE), ecosystem respiration (Reco), and gross primary production (GPP), over the course of the study from 14th of October 2014 to 15th of May 2015, for each prolonged dry season and N addition treatment (control and N addition). (a) no prolonged dry season (watering started on the 14th of October); (b) 50-day prolonged dry season (watering started on the 3rd of December); (c) 100-day prolonged dry season (watering started and the 20th of January).

season, were concurrent with changes in LAI (Table S2). However, for the no extended drought treatment NEE_{max} flux rates did not correlate with LAI.

3.5. Community productivity and composition

Extending the dry season induced in a significant decrease of total aboveground biomass ($F_{2,47} = 57.61$, $P < 0.001$). However, there was a significant interaction between prolonged dry season and sampling month ($F_{2,47} = 13.56$; $P < 0.001$). In April, aboveground biomass differed significantly between all levels of prolonged dry season, while in May, differences were only significant between no prolonged dry season and 100-day prolonged dry season (average 20.5% lower) (Fig. 4a, c). Total aboveground biomass was not affected by N addition (Fig. 4a, c). Between April and May there was an overall strong productivity recovery for 50 and 100-day prolonged dry season treatments (Fig. 4a, c).

Regarding different functional group behavior, forbs were the most abundant functional group in all six treatments (78.85% ± 1.11, average across all treatments, but see Nogueira et al., 2017 for additional details), followed by graminoids (15.42% ± 0.86) and legumes (5.74% ± 0.68) (Fig. 4b, d). Extending the dry season decreased relative abundance of forbs ($F_{2,47} = 0.01$, $P < 0.001$) (Fig, 4b, d), whereas relative abundance of graminoids and legumes increased ($F_{2,47} = 6.45$, $P = 0.004$ and $F_{2,47} = 80.74$, $P < 0.001$, respectively) (Fig. 4b,d). Relative abundance of graminoids was favored by the 50-day prolonged dry season (Fig. 4b, d). Functional groups were unresponsive to N addition.

4. Discussion

Our results highlight the negative effects of a prolonged dry season and delayed onset of autumn rains on CO_2 and water flux rates and productivity of an annual Mediterranean grassland community. We found that a prolonged dry season led to lower total CO_2 fluxes, although the negative effects were nearly compensated by N addition. These results stress the importance of considering interacting global change drivers on the functioning of Mediterranean grasslands.

In Mediterranean grasslands, the length of the growing season plays an important role in ecosystem CO_2 uptake (Jongen et al., 2011; Ma et al., 2007; Piayda et al., 2014). This is linked to the timing of first rain events in autumn but also to the rapid reduction in soil moisture and increase in high temperatures that occurs usually between May and early June (Aires et al., 2008a; Cerasoli et al., 2018; Ma et al., 2007; Xu and Baldocchi, 2004). For example, in a Mediterranean grassland in California, earlier autumn rains increased winter GPP by ~90 g C m^{-2} due to an earlier start of grass germination, even though the total precipitation was low (Xu and Baldocchi, 2004). Also, Piayda et al. (2014) reported that GPP of the herbaceous understory of a savannah woodland in Portugal was reduced by 53% due to one-month delay of the first autumn rains and a reduction in winter precipitation. As a consequence, and in agreement with our results, these authors observed a substantial delay in seed germination and leaf area growth and a later start of CO_2 sequestration, with significant negative consequences for the annual carbon balance. Indeed, favorable growing conditions at the beginning of our experiment gave C0 and N0 communities an important development advantage in LAI. Increased leaf area stimulated by mild temperatures and water availability (Xu and Baldocchi, 2004), allowed a rapid dominance of GPP over Reco, reinforcing the importance of autumn growth for total CO_2 fluxes.

An early and dense grassland cover development has not only positive effects on total CO_2 fluxes but may also additionally have strong impacts on soil water relations, such as reduction of soil evaporation and increased rain infiltration, especially under strong rain events (e.g. Dubbert et al., 2014a) that are typical of Mediterranean climates.

Variations in GPP_{max}, NEE_{max}, $Reco_{max}$ and ET_{max} flux rates throughout the growing season were highly correlated with seasonal measured differences in LAI, as the potential to intercept light for photosynthesis or for water vapour exchanges are limited by leaf area and physiological capacity (Aires et al., 2008a, 2008b; Jongen et al., 2011). Also respiration substrates (leaves, stems and roots) and litter input to the soil usually increase with LAI (Reichstein et al., 2002). Although, LAI has a large effect on ecosystem CO_2 exchange, leaf-level photosynthetic capacity also varies during the season (Huxman et al., 2004c), which may explain the decrease in GPP flux rates in mid-May in the no prolonged dry

Table 2
Total cumulative net ecosystem exchange (NEE), ecosystem respiration (Reco) and gross primary production (GPP), from 14th of October 2014 to 15th of May 2015, for each prolonged dry season treatment and N addition treatment. C - represents no added nitrogen (control), N - represents added nitrogen, the number next to the letters represents number of days of prolonged dry season. Watering started on the 14th of October for 0-day prolonged dry season; on the 3rd of December for 50-day prolonged dry season; and 20th of January for 100-day prolonged dry season.

	C0	N0	C50	N50	C100	N100
NEE (g CO_2 m^{-2})	−241.58	−225.25	−238.66	−303.15	−181.92	−253.41
Reco (g CO_2 m^{-2})	352.79	384.03	232.80	340.90	205.32	215.25
GPP (g CO_2 m^{-2})	594.36	609.28	471.46	644.05	458.73	468.66

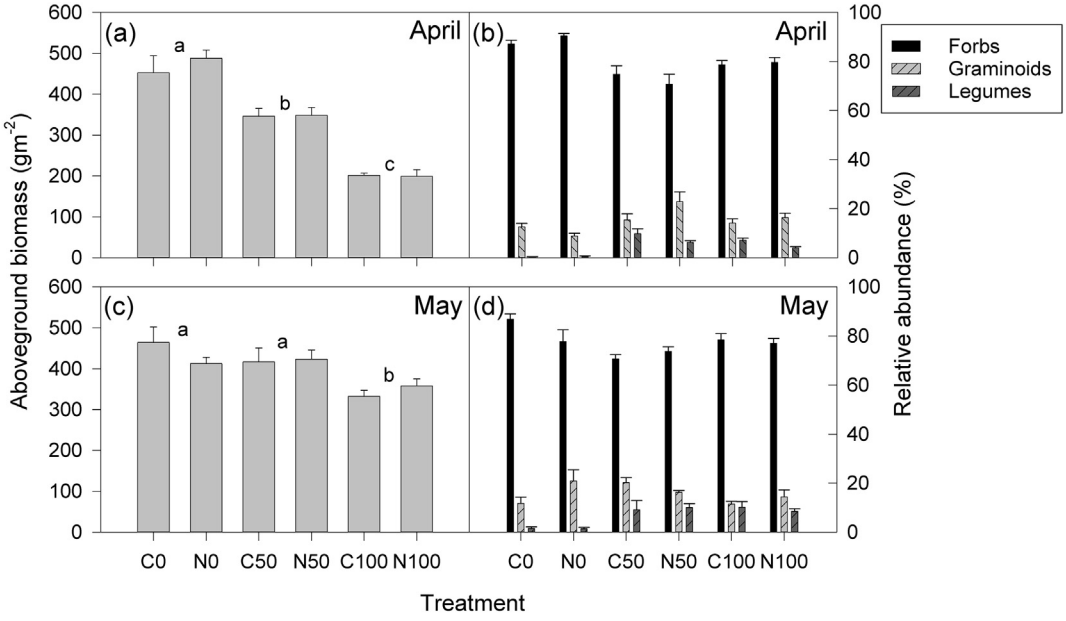

Fig. 4. Aboveground biomass in April (a) and in May (c) and relative abundance of functional groups (forbs, graminoids and legumes) in April (b) and May (d), for each prolonged dry season treatment and N addition treatment. C - represents no added nitrogen (control), N - represents added nitrogen, the number next to the letters represents number of days of prolonged dry season. Watering started on the 14th of October for 0-day prolonged dry season; on the 3rd of December for 50-day prolonged dry season; and 20th of January for 100-day prolonged dry season. Bars show mean ± SE, $n = 4$. Different letters indicate significantly different means between prolonged dry season treatments (Tukey method, $P < 0.05$).

season treatment, as these communities commence to senesce (Nogueira et al., 2017), compared to the younger communities in the 100-day prolonged dry season treatment. Changes in grassland community composition could also have affected the GPP_{max} flux rates. For example, the higher proportion of graminoids and legumes, that generally have an overall higher specific leaf area (higher metabolic rates) compared to rosette species (e.g. forbs) (Ansquer et al., 2009; Westoby et al., 2002; Nogueira et al., 2018) may further have contributed to the faster response in GPP_{max} flux rates in the 50 and 100-day prolonged dry season treatments, which translated into an important aboveground biomass increment from April to May. In fact, the functional group composition of these grasslands is highly dynamic and responsive to environmental conditions and, specifically to drought (e.g. Espigares and Peco, 1995; Dubbert et al., 2014b; Nogueira et al., 2017, 2018; Peco et al., 1998).

In Mediterranean ecosystems, the increase in soil moisture, with first autumn rains, leads to a burst of decomposition, mineralization and release of inorganic N and CO_2 (Huxman et al., 2004c; Jarvis et al., 2007; Unger et al., 2010, 2012). Also, the longer the previous drought period, the greater the decomposition and mineralization rates on rewetting (Jarvis et al., 2007; Unger et al., 2010). In annual dominated grasslands, this N input from mineralization with first autumn rains is most probably lost through leaching, as annuals have not yet germinated and no other plant life forms generally exist (e.g. perennials) that can use the available N (Joffre, 1990). Subsequent ecosystem CO_2 uptake will depend on ensuing precipitation, germination and photosynthesis, as well as on the magnitude of Reco, through its response to temperature and soil water (e.g. Jongen et al., 2011).

GPP increased more strongly along the growing season than did Reco as GPP follows the seasonal dynamics of light and air temperature (Gilmanov et al., 2007). Slower increment of Reco throughout the growing season can be explained by the fact that Reco, which includes both plant and soil respiration, depends not only on leaves and respiring supporting tissues but also on available substrates, such as root exudates, supplied by photosynthesis (Aires et al., 2008a; Li et al., 2017; Reichstein et al., 2002, 2003) that increase with LAI and GPP (Reichstein et al., 2003). Differences in the proportion of functional groups between treatments may also have influenced Reco. Legume species, for instance, have a positive effect on soil respiration (Li et al., 2012; Tjoelker et al., 2005) which might have contributed to similar Reco flux rates in February between no prolonged and 50- day prolonged dry season, despite the latter having significantly lower LAI.

As our community was N limited, we hypothesized that the positive relationship between N addition, photosynthetic rate and LAI (Niu et al., 2009, 2010, Pérez-Priego et al., 2015a) would attenuate the negative effects of a prolonged dry season on GPP. Also, expected stimulation of leaf photosynthesis would provide more substrate for soil and plant respiration, leading to an increase in Reco under N addition (Niu et al., 2010), although contradictory or null results have also been shown (e.g. Bubier et al., 2007; Janssens et al., 2010). As for a study performed in the same grassland field at CL (Cerasoli et al., 2018), we did not find a clear effect of N addition on CO_2 flux rates under control conditions. However, N addition substantially attenuated the negative effect on CO_2 fluxes induced by the prolonged dry season treatments. The positive effect of N addition on NEE suggests that under the occurrence of a prolonged dry season, as predicted by climate models, N deposition may increase CO_2 sequestration in Mediterranean grasslands.

We found that late arrival of autumn rains affected the establishment of herbaceous species, with consequences for community composition and biomass production (Espigares and Peco, 1995; Miranda, de Dios, et al., 2009; Nogueira et al., 2017, 2018). Accordingly, we observed differences in relative abundance of functional groups and a significant negative effect on aboveground biomass. Cooler temperatures occurred in December and January when first watering of the 50 and 100-day drought treatments took place, which favored the establishment of legumes species, whereas forb species, some of which can have large biomass (e.g. *Echium plantagineum*; *Raphanus raphanistrum*) were favored by early autumn rains (Del Pozo and Aronson, 2000; Espigares and Peco, 1993; please see Nogueira et al., 2017, 2018 for details).

In our study, grassland community productivity was 464.34 $g \cdot m^{-2} \pm$ 37.71 (C0) in May which was well within the range of grassland productivity in the field site at CL. The growing season productivity of the field CL grassland ranged from 473.19 $g \cdot m^{-2}$, in a wet year to 234.28 $g \cdot m^{-2}$ in a very dry year. In our study, higher total GPP with N addition did not

translate into increased aboveground biomass. A lack of productivity response to N addition, within the predicted N deposition values for the Mediterranean Basin (10 and 20 kg N ha^{-1} $year^{-1}$), was also found by Ochoa-Hueso and Stevens (2015), in a semiarid shrubland in central Spain. The authors observed species-specific biomass responses and a significant change in plant community composition, with responses to N occurring approximately 2.5 years after N addition and controlled by precipitation and P availability. In our study, despite changes in functional group composition with a prolonged dry season, forbs still represented ca. 79% of our grassland community. Forbs, included species such as the *Echium plantagineum* (Nogueira et al., 2017) that have N conserving strategies, such as low N tissue concentration and low relative growth rates (e.g. low specific leaf area) (Ansquer et al., 2009; Craine et al., 2001) which might, at least partially, explain the lack of strong response to increased N availability (Cleland and Harpole, 2010). Responses to N addition must be considered in the context of multiple interacting factors (Bobbink et al., 2010; Cleland and Harpole, 2010). The increase in growth might be limited when soil chemical properties (e.g. pH), other nutrients, such as P and base cations, become more limiting than N (e.g. Bobbink et al., 1998). In fact, our field grassland in CL is also P limited (personal observation). Life-stage responses of individual species to N addition may also mask overall grassland responses (Tulloss and Cadenasso, 2016). Additionally, as we sieved the soil used in this experiment, we might have increased mineralization in all treatments which probably led to an underestimation of N effects.

5. Conclusions

Our results showed that a prolonged dry season had a significant impact on an annual Mediterranean grassland community leading to changes in community composition, decreased total CO_2 fluxes and aboveground productivity due to a shortening of the effective growing season. We also found that N addition attenuated the negative effects of a prolonged dry season on ecosystem CO_2 fluxes. This is especially relevant in Mediterranean climates with marked precipitation seasonality where droughts significantly reduce CO_2 sequestration and where prolonged summer droughts are projected to occur. Differences in aboveground biomass observed in early spring, between prolonged dry season treatments, anticipate that resilience of Mediterranean grasslands might be negatively affected by the combination of a late rain onset and the forecasted decrease in spring precipitation. Our results emphasize the importance to consider interacting global change drivers on the functioning and resilience of Mediterranean grassland ecosystems and point towards the complexity of responses of such diverse ecosystems to these drivers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.11.091.

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